

Structure of the Bivalve Rectum

II. Notes on Cell Types and Innervation

BY

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(Plates 45 to 48)

INTRODUCTION

RECENTLY, THERE HAS BEEN increasing interest in the pharmacology (GREENBERG & JEGLA, 1963; GREENBERG, 1966; PHILLIS, 1966) and physiology (PROSSER, NYSTROM & NAGAI, 1965; NYSTROM, 1967) of the bivalve rectum as an example of an invertebrate organ with smooth muscle. While these studies have all been concerned with the innervation of the nonstriated muscle of the rectum, the histology of this structure has been but briefly noted (GREENBERG & JEGLA, 1962; PHILLIS, 1966).

Although the structure of molluscan muscles has been intensively studied (see HANSON & LOWY, 1960, 1965; HOYLE, 1964), the bulk of these investigations have concerned skeletal muscles such as shell adductors and byssus retractors. Consequently, with the exception of work on heart (reviewed by HILL & WELSH, 1966; NISBET & PLUMMER, 1966), visceral musculature has largely been ignored.

We have shown that the gross histology of bivalve rectums varies markedly from species to species (JEGLA

& GREENBERG, 1968). Also, GREENBERG (1966) described striking pharmacological differences between the acetylcholine responses of two intrageneric species. In the present study we have looked for specific differences in the cellular elements which might be correlated with gross structural or pharmacological differences. In addition we have made observations of the pattern of innervation of the rectal musculature of some species. Finally, we have compared the structure of the rectal muscle fibers with those of the heart and other molluscan muscles.

MATERIALS AND METHODS

The species of bivalve mollusks and methods used in this study were identical to those previously reported (JEGLA & GREENBERG, 1968). In addition, for observation of nerves, we have used a modification of Bodian's silver staining technique as well as the method of KOELLE (1951) for acetylcholinesterase.

Explanation of Plate 45

Figure 1: A transverse section through the wall of a *Mya arenaria* rectum showing the columnar epithelium, a layer of collagen fibers and circular muscle arranged in bundles.

C - columnar epithelium
CTF - connective tissue fibers
M - muscle cell

Figure 2: *Quadrula quadrula* (RAFINESQUE, 1820). Muscle is arranged in bundles and embedded in a network of connective tissue.
Figure 3: *Amblema plicata* RAFINESQUE, 1820. Connective tissue in the typhlosole.

CM - circular muscle
F - fibroblast cell
P - phagocyte

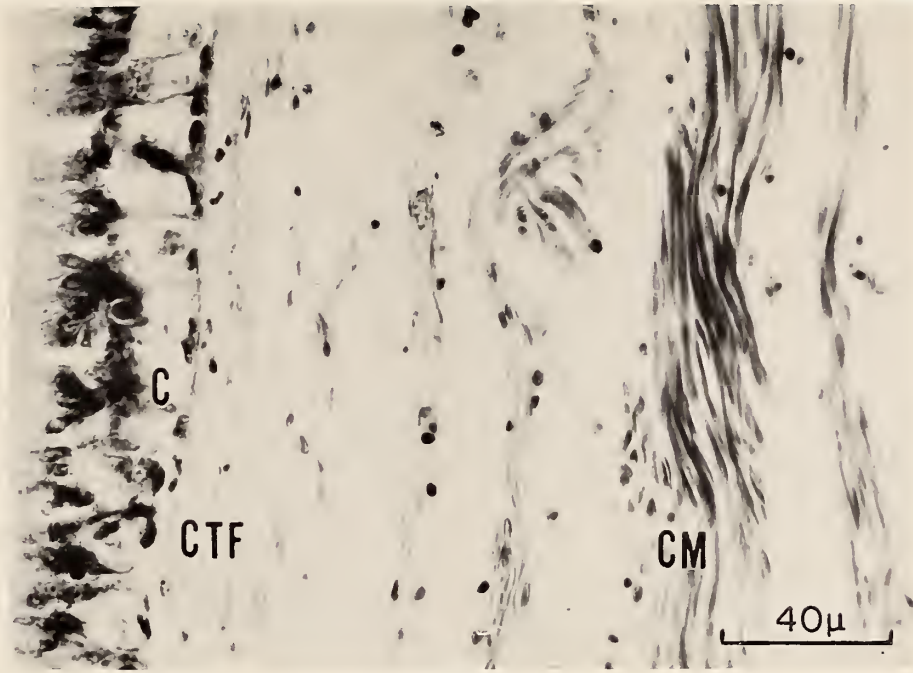


Figure 1



Figure 2

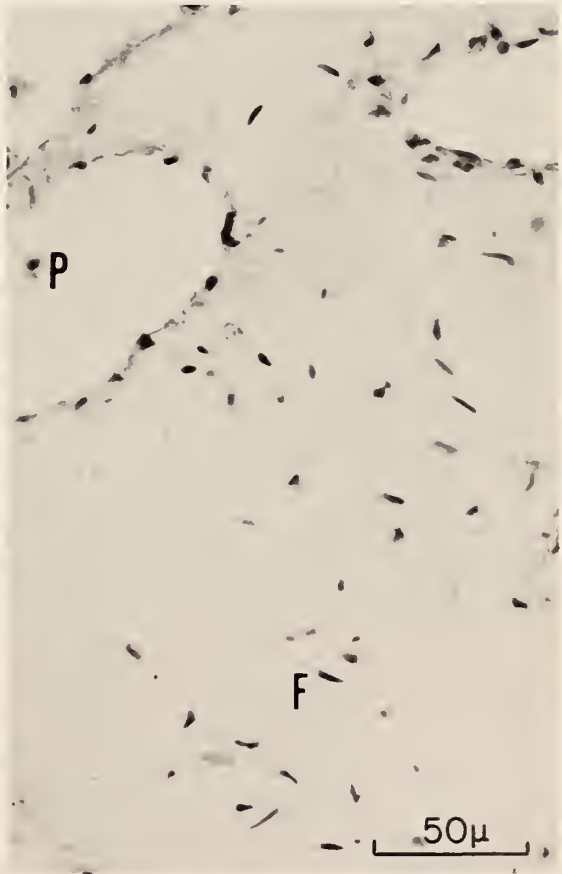


Figure 3



RESULTS

The wall of the bivalve rectum is composed of an inner columnar epithelium, connective tissue, and muscle layers (Figure 1). The epithelium is separated from the more peripheral elements by a basement membrane. These elements, as well as innervation of the muscle are discussed below.

Epithelial Cells

Details of the columnar epithelial cells lining the digestive tract lumen in *Anodonta cellensis* SCHRÖTER, 1779 (see GUTHEIL, 1912) and *Ostrea edulis* LINNAEUS, 1758 (see YONGE, 1926) have been described. Only a few additional comparative observations will be given here. These cells are much higher than wide but their average height varies depending, not only on the species, but also on the size of the individual animal. The smallest cells were found in *Nucula* (7.5μ) and the largest in *Mytilus californianus* CONRAD, 1837 (115μ). In a very small *Actinonaias carinata* (BARNES, 1823) the average cell height was about 26μ , while in a large specimen of the same species, it was about 74μ . In some species, height of the cells may vary greatly around the periphery of the lumen; this is observed in conjunction with ridges and furrows, and with typhlosoles. In *Mytilus edulis* LINNAEUS, 1758 the columnar cells are twice as large on the ventral side of the rectum as they are on the dorsal side (see Part I, plate 37, fig. 3). The reverse situation occurs in *Actinonaias* (see Part I, plate 39, fig. 7) and other unionids. Furthermore, the cytoplasm of the small cells bordering the unionid typhlosole stains more intensely than that of the taller cells on the opposite side of the rectum.

DAKIN (1909) thought that cilia are poorly developed in the rectum of *Pecten maximus* (LINNAEUS, 1758), and WHITE (1937) reported that cilia are long but scantily developed in the rectum of *Mytilus edulis*. However, rectal cilia were fairly abundant in all the species we studied. The mean length of the cilia in most species was between 6μ and 12μ . Conspicuously smaller cilia were observed in *Nucula* and *Dinocardium robustum* (LIGHTFOOT, 1786) [about 4μ], and larger cilia in *Chlamys* and *Mya arenaria* LINNAEUS, 1758 (15μ to 16μ).

Mucous cells and phagocytes occur in the columnar epithelial layer and were described by YONGE (1926) in *Ostrea*, and by GUTHEIL (1912) in *Anodonta*. The fibrous nature of the underlying basement membrane is easily observed in *Atrina rigida* (LIGHTFOOT, 1786) (Plate 46, Figure 4). There is some small interspecific variation in thickness of this membrane but in all species

it is thin (1μ to 5μ). The outer surface of the rectum is covered by a very thin epithelium which is observed only with difficulty.

Connective Tissue Fibers and Cells

The numerous, randomly oriented fibers in the rectum have the staining properties of collagen (Plate 45, Plate 46, Fig. 4). They are considerably smaller than the average muscle fiber, and are less than 1μ in diameter. Their length is not known because they are contorted and their ends could not be observed in histological sections. This feature is shared with the collagen fibers in vertebrate connective tissue. A reticulum of very small fibers is frequently observed around the individual muscle fibers.

The prominent cellular elements of the connective tissue are phagocytes, fibroblasts, and vesicular cells. Phagocytes are numerous among the columnar cells, throughout the wall of the rectum and in the circulation. These cells were described extensively by GUTHEIL (1912) and YONGE (1926). While the amount of cytoplasm is variable, the nucleus is spherical, small (2.5μ to 4μ in diameter), and hyperchromatic (Plate 45, Fig. 3); these characteristics serve to distinguish a phagocyte from any other cell in the rectum.

Fibroblast cells are a universal and common component of the connective tissue. They are fusiform or stellate-shaped, with fibrous processes extending from the cell body. The fusiform type is 2.5μ by 4 to 12μ in size; it has one or two fibrous extensions and the nucleus occupies most of the cell body (Plate 45, Figure 3). The stellate cells average 5μ by 7μ ; they have three or more long fibrous extensions and a small spherical nucleus. Fibroblasts are best observed in typhlosoles, since they are filled, principally, with fibrous connective tissue.

The rectums of a few species have vesicular cells that are spherical to oval in shape. These are the largest connective tissue cells in the rectum (up to 13μ by 40μ); they resemble those cells surrounding the digestive tract in the visceral mass. Generally, vesicular cells are empty after fixation; the sparse cytoplasm and small nucleus are located centrifugally.

Muscle

At the light microscope level all of the muscle fibers in the bivalve rectum are nonstriated. They are spherical or oval in transverse section (Plate 46, Figure 5), and have a diameter of 1μ to 6μ ; the larger diameters may be the result of contraction. The fibers are very long, at least 170μ , but we were unable to determine their exact length. The muscle cells are uninucleate; the nucleus is large, oval, and with its major axis parallel to the longi-

tudinal axis of the fiber (Plate 46, Figure 5). In all species the muscle nuclei can be distinguished from those of fibroblasts by the larger size and lighter staining quality of the former.

The fibers are arranged in bundles in *Mya* (Plate 45, Figure 1) and the family Unionidae (Plate 45, Figure 2), but there is little indication of such grouping in Cardidae or Mactridae which have the muscle fibers packed closely together throughout the area of their occurrence in the rectum. In those species with a scarcity of muscle, and in the Veneridae, the muscle cells are generally not organized into bundles but lie embedded as individual units in the meshwork of connective tissue fibers and cells (Plate 47, Figure 6).

HANSON & LOWY (1957) distinguished three kinds of molluscan nonstriated muscle on the basis of the distribution, in the cell, of myofibrils, the course (*e. g.* helical or straight) of the myofibrils, and the appearance of "contraction bands" during excessive shortening of the muscle. We have examined these characteristics.

Myofibrils are not restricted to the periphery of the fiber in the rectum. While this is especially clear in transverse sections of *Dinocardium* muscle (Plate 46, Figure 5), an even distribution of fibrils in the sarcoplasm has been found in all of the species examined.

Rectums of *Mercenaria mercenaria* (LINNAEUS, 1758), fixed either while tied at an extended length, or while in 5-hydroxytryptamine contracture, were compared. In the stretched rectum, the smallest fibers have the classic appearance of vertebrate smooth muscle; no myofibrils are visible. But the larger fibers have longitudinal fibrils lying parallel to the long axis; they were not helically arranged. We were unable to determine the length of these fibrils. In *Mercenaria* rectums highly contracted by 5-hydroxytryptamine, many of the fibers appear to have contraction bands which cross each other at angles of up to 20° to the fiber axis, producing the classical double-oblique, or diamond lattice pattern. Many of our preparations from other species also showed this pattern following fixation or 5-hydroxytryptamine contracture (Plate 46, Figure 5).

Comparison of Heart and Rectal Muscle

The nonstriated muscle fibers of the heart of *Mercenaria* are different from those of the rectum in that the myofibrils are peripherally disposed (Plate 47, Figure 7). This difference in the distribution is probably not due to artifact since both tissues were necessarily fixed, embedded and stained together. However, NISBET & PLUMMER (1966) examined the fine structure of the heart of achatinid snails and found that the contractile elements were centrally disposed in the muscle cells, occupying about $\frac{2}{3}$ of the cross-sectional area.

The myofibrils of the *Mercenaria* heart appear to spiral and helical "contraction bands" were observed (Plate 47, Figure 8). Often the fibrils are observed to cross at acute angles, producing the classic diamond lattice pattern of MARCEAU (1905, 1909). A reticulum of very fine connective tissue fibers surrounding individual muscle fibers was often observed. Larger collagen fibers and fibroblast cells are found between the heart muscle cells. Nuclei are centrally placed and appear to be more numerous in heart muscle; perhaps the fibers are shorter, or syncytial as was proposed for the *Mercenaria* heart (LOVELAND, 1963).

Nervous Tissue

Intrinsic nerve fibers and cells have not often been demonstrated in bivalve muscles. BRÜCK (1914) illustrated small nerve fibers ending in motor end plates on muscle cells. BOWDEN (1958), using the cholinesterase technique of KOELLE (1951), demonstrated small nerve fibers and motor end plate-like structures in the fast part of the adductor muscles of *Anodonta* and *Unio*; in other muscles studied, only free terminations were observed. BOWDEN also reported ganglion cell-like structures and nerve fibers in the slow parts of bivalve adductors. PHILLIS (1966) observed small nerve fibers and a ganglion cell in the rectum of *Tapes waltlingi* IREDALE, 1958.

Occasionally we have seen ganglion cells and nerve fibers in the rectum of *Dinocardium*. The nerve fibers bifurcate near their ends and terminate on different

Explanation of Plate 46

Figure 4: *Atrina rigida* (LIGHTFOOT, 1786). Shows the fibrous nature of the basement membrane.

Figure 5: *Dinocardium robustum* (LIGHTFOOT, 1786). Muscle cells of the rectum in longitudinal and transverse section. Note the

BM - basement membrane

C - columnar epithelium

N - nucleus

distribution of myofibrils in the transverse section and the occurrence of the diamond lattice pattern in the longitudinal sections of muscle cells.

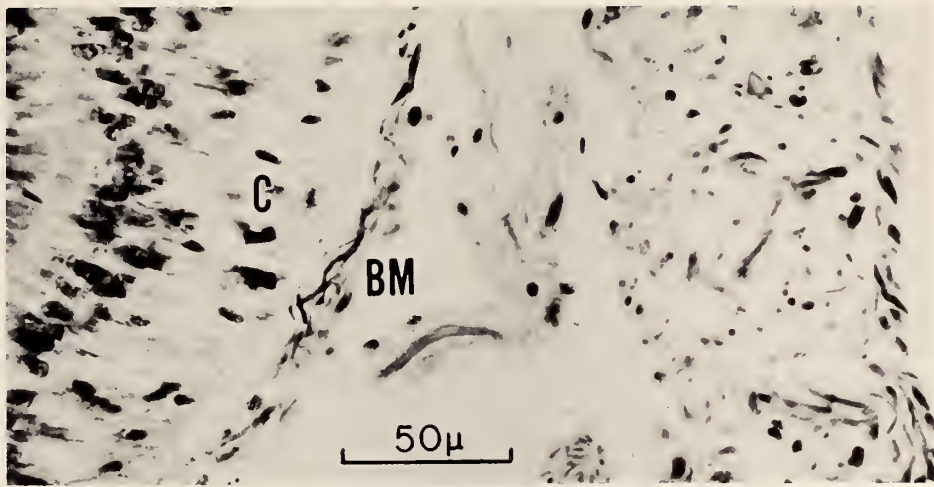


Figure 4

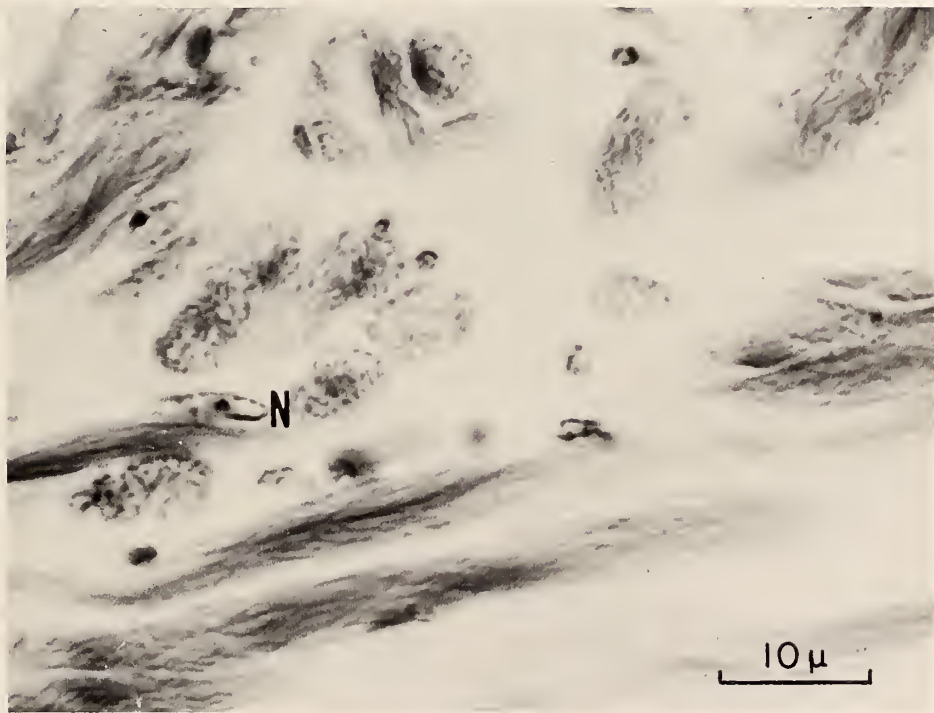


Figure 5